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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Stefan KAPPELER	)	Art Unit:
U.S. App. No.: 09/985,936	)	Washington, D.C.
National Filing Date:11/06/2001	)	February 20, 2002
For: METHOD OF PRODUCING NON-BOVINE CHYMOSIN AND USE HEREOF	)	Docket No.:KAPPELER=1A

PRELIMINARY AMENDMENT

Honorable Commissioner for Patents and Trademarks
Washington, D.C. 20231

Sir:

Prior to examination upon the merits, kindly amend as follows:

IN THE CLAIMS

Please cancel claims 1, 17-34, and 41-48.

Please amend claims 2, 4, 5, 7, 8, 10, 13, 14, 35, 37, and 38 to read as follows:

2(amended). A method according to claim 51 wherein the coding sequence is derived from a mammalian species selected from the group consisting of a ruminant species, a *Camelidae* species, a porcine species, and *Equidae* species and a primate species.

4(amended). A method according to claim 51 wherein the coding sequence for pre-prochymosin, prochymosin and chymosin is isolated or derived from *Camelus dromedaries*.

5(amended). A method according to claim 51 wherein the nucleic acid sequence codes for a fusion protein comprising pre-prochymosin, prochymosin or chymosin.

7(amended). A method according to claim 51 wherein the pre-prochymosin, prochymosin or chymosin, or a fusion protein thereof, is secreted over the host cell membrane.

8(amended). A method according to claim 51 wherein the expression vector is derived from pGAMpR as described in Ward et al., 1990 by substituting the coding sequence of that vector for bovine prochymosin with a coding sequence for the non-bovine pre-prochymosin, prochymosin or chymosin.

10(amended). A method according to claim 51 wherein the transformed host cell is selected from the group consisting of a bacterial cell, a fungal cell, a yeast cell, a mammalian cell, an insect cell and a plant cell.

13(amended). A method according to claim 51 wherein the yield of pre-prochymosin, prochymosin or chymosin milk clotting activity is at least 25 % higher than the yield of bovine pre-prochymosin, prochymosin or chymosin milk clotting activity obtained when using, under identical production conditions, the same expression vector, but with a coding sequence for bovine pre-prochymosin, prochymosin or chymosin in place of the coding sequence for the non-bovine pre-prochymosin, prochymosin or chymosin.

14(amended). A method according to claim 51 comprising, as a further step, that the harvested pre-prochymosin, prochymosin or chymosin is subjected to a deglycosylation treatment.

35. (amended) A composition comprising a non-bovine pre-prochymosin, prochymosin or chymosin produced by the method of claim 51.

37(amended). A composition according to claim 35 comprising pre-prochymosin, prochymosin or chymosin derived from the group consisting of a *Camelidae* species, a buffalo species, an ovine species or a caprine species.

38(amended). A method of manufacturing cheese, comprising adding a milk clotting effective amount of the composition according to claim 35 to milk and carrying out appropriate further cheese manufacturing steps.

Please add claims 49-51.

49(new). A DNA construct, the nucleic acid sequence of which comprises a coding sequence coding for an expressible protein which is (I)(a) a non-bovine pre-prochymosin, prochymosin, or chymosin or (b) a fusion protein comprising a core protein which is a pre-prochymosin, prochymosin or chymosin, and cleavable to release said core protein; and

(II) appropriate expression signals, operably linked to said coding sequence, permitting the protein to be expressed in a host cell.

50(new). A host cell transferred with the DNA construct of claim 49, said cell being one in which said expression signals are operable.

51(new). A method of producing a protein of interest selected from the group consisting of non-bovine pre-prochymosin, prochymosin, and chymosin which comprises

providing a host cell according to claim 50,

cultivating said host cell under conditions where said expressible protein is expressed,

if said expressible protein is a fusion protein, cleaving it to release said protein of interest, and

harvesting the protein of interest.

REMARKS

The above amendments to the claims are being made in order to eliminate multiple dependency and for the purpose of reducing the filing fee. Please enter this amendment prior to calculation of the filing fee in this case.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**Version with Markings to Show Changes Made**."

Favorable consideration and allowance are earnestly solicited.

Respectfully submitted,
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VERSION WITH MARKINGS TO SHOW CHANGES MADE

Claims 1, 17-34, and 41-48 have been cancelled.

2. A method according to [claim 1] claim 51 wherein the coding sequence is derived from a mammalian species selected from the group consisting of a ruminant species, a *Camelidae* species, a porcine species, and *Equidae* species and a primate species.

4. A method according to [claim 1] claim 51 wherein the coding sequence for pre-prochymosin, prochymosin and chymosin is isolated or derived from *Camelus dromedaries*.

5. A method according to [claim 1] claim 51 wherein the nucleic acid sequence codes for a fusion protein comprising pre-prochymosin, prochymosin or chymosin.

7. A method according to claim 51 [any of claims 1-6] wherein the pre-prochymosin, prochymosin or chymosin, or a fusion protein thereof, is secreted over the host cell membrane.

8. A method according to claim 51 [claim 1] wherein the expression vector is derived from pGAMpR as described in Ward et al., 1990 by substituting the coding sequence of that vector for bovine prochymosin with a coding sequence for the non-bovine pre-prochymosin, prochymosin or chymosin.

10. A method according to claim 51 [any of claims 1-9] wherein the transformed host cell is selected from the group consisting of a bacterial cell, a fungal cell, a yeast cell, a mammalian cell, an insect cell and a plant cell.

13. A method according to claim 51 [any of claims 1-12] wherein the yield of pre-prochymosin, prochymosin or chymosin milk clotting activity is at least 25 % higher than the yield of bovine pre-prochymosin, prochymosin or chymosin milk clotting activity obtained when using, under identical production conditions, the same expression vector, but with a coding sequence for bovine pre-prochymosin, prochymosin or chymosin in place of the coding sequence for the non-bovine pre-prochymosin, prochymosin or chymosin.

14. A method according to claim 51 [any of claims 1-13] comprising, as a further step, that the harvested pre-prochymosin, prochymosin or chymosin is subjected to a deglycosylation treatment.

35. A composition comprising a non-bovine pre-prochymosin, prochymosin or chymosin produced by the method of claim 51 [any of claims 1-16].

37. A composition according to claim 35 [claim 35 or 36] comprising pre-prochymosin, prochymosin or chymosin derived from the group consisting of a *Camelidae* species, a buffalo species, an ovine species or a caprine species.

38. A method of manufacturing cheese, comprising adding a milk clotting effective amount of the composition according to claim 35 [claim any of claims 35-37] to milk and carrying out appropriate further cheese manufacturing steps.

Claims 49-51 have been added.